

TRANSMITTAL LETTER THE
UNITED STATES RECEIVING OFFICE

DATE	January 2004
INTERNATIONAL APPL. NO.	US03/18046
ATTY DOCKET NO.	16325-136PC

10/516780

I. Certification under 37 CFR 1.10 (if applicable)	
EV 330 850 358 US	5 January 2004
Express Mail mailing number	Date of Deposit

II. <input type="checkbox"/> New International Application									
Title	Earliest priority date (Day/Month/Year)								
<p>SCREENING DISCLOSURE INFORMATION: In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):</p> <p>A. <input type="checkbox"/> The invention disclosed was not made in the United States. B. <input type="checkbox"/> There is no prior U.S. application relating to this invention. C. <input type="checkbox"/> The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. (NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claims for priority).</p> <table border="1"> <tr> <td>application no.</td> <td></td> <td>filed on</td> <td></td> </tr> <tr> <td>application no.</td> <td></td> <td>filed on</td> <td></td> </tr> </table> <p>D. <input type="checkbox"/> The present international application <input type="checkbox"/> contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C above. The additional subject matter is found on pages: _____ and <input type="checkbox"/> DOES NOT ALTER <input type="checkbox"/> MIGHT BE CONSIDERED TO ALTER the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 USC 181 and 37 CFR 5.1. See 37 CFR 5.15.</p>		application no.		filed on		application no.		filed on	
application no.		filed on							
application no.		filed on							

III. <input type="checkbox"/> A Response to an Invitation from the RO/US. The following document(s) is (are) enclosed:																							
<p>A. <input type="checkbox"/> A Request for an Extension of Time to File a Response. B. <input type="checkbox"/> A Power of Attorney (General or Regular) C. <input type="checkbox"/> Replacement pages:</p> <table border="1"> <tr> <td>pages</td> <td></td> <td>of the request (PCT/RO/101)</td> <td>pages</td> <td></td> <td>of the figures</td> </tr> <tr> <td>pages</td> <td></td> <td>of the description</td> <td>pages</td> <td></td> <td>of the abstract</td> </tr> <tr> <td>pages</td> <td></td> <td>of the claims</td> <td></td> <td></td> <td></td> </tr> </table> <p>D. <input type="checkbox"/> Submission of Priority Documents</p> <table border="1"> <tr> <td>Priority document</td> <td></td> <td>Priority document</td> <td></td> </tr> </table> <p>E. <input type="checkbox"/> Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex</p>		pages		of the request (PCT/RO/101)	pages		of the figures	pages		of the description	pages		of the abstract	pages		of the claims				Priority document		Priority document	
pages		of the request (PCT/RO/101)	pages		of the figures																		
pages		of the description	pages		of the abstract																		
pages		of the claims																					
Priority document		Priority document																					

IV. <input type="checkbox"/> A Request for rectification under PCT 91	<input type="checkbox"/> A Petition	<input checked="" type="checkbox"/> A Sequence Listing, Statement, Diskette
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V. <input checked="" type="checkbox"/> Other (please specify):	<input checked="" type="checkbox"/> Postcard	<input checked="" type="checkbox"/> Chapter II Demand	<input checked="" type="checkbox"/> Letter to USPTO Officer
<input checked="" type="checkbox"/> Article 34 Amendment with seven (7) substitute pages 6, 25, 45, 64, 65, 70 and 80			

The Commissioner is hereby authorized to charge any additional fees associated with this paper or during the pendency of this application, or credit any overpayment, to Deposit Account No. 20-1430.

The person signing this form is the	<input type="checkbox"/> Applicant	Jean M. Lockyer
	<input checked="" type="checkbox"/> Attorney/Agent (Reg. No.) 44,879	Typed name of signer
	<input type="checkbox"/> Common Representative	Signature

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and
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5 January 2004

VIA EXPRESS MAIL, WITH RETURN POSTCARD ENCLOSED

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
PO Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US03/18046
Title: METHODS OF DIAGNOSING & TREATING DIABETES AND INSULIN
RESISTANCE
Applicant: METABOLEX, INC. *et al.*
International Filing Date: 5 June 2003
Express Mail Label No.: EV 330 850 358 US
Date of Mailing: 5 January 2004
Our File No.: 16325-136PC

Dear Officer:

Enclosed are the Chapter II Demand and seven (7) substitute specification pages 6, 25, 45, 64, 65, 70 and 80 submitted as an Article 34 Amendment for the above-referenced patent application. The only changes were insertions of SEQ ID: NOs. and corrections of typographical errors that do not include matter which goes beyond the disclosure in the international application as filed.

Thank you for your attention to this matter.

Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP

Jean M. Lockyer
Reg. No. 44,879

JML/nan

Enclosures:

Chapter II Demand
Seven (7) substitute specification pages 6, 25, 45, 64, 65, 70 and 80
Sixty-two (62) pages of Sequence Listing
Diskette and Statement
Transmittal Letter and Postcard

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

DEMAND

CHAPTER II

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference
International application No.	International filing date (day/month/year)	16325-136PC
PCT/US03/18046	05 June 2003 (05.06.03)	(Earliest) Priority date (day/month/year)
Title of invention		05 June 2002 (05.06.02)
METHODS OF DIAGNOSING & TREATING DIABETES AND INSULIN RESISTANCE		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		Telephone No.:
METABOLEX, INC.		510.293.8800
3876 Bay Center Place		Facsimile No.:
Hayward, California 94545		510.293.9090
United States of America		Teleprinter No.:
		Applicant's registration No. with the Office
State (that is, country) of nationality:	State (that is, country) of residence:	
US	US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		
ALLAN, Bernard		
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United States of America		
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IE	US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		
GREGOIRE, Francine		
1044 Carol Lane		
Lafayette, California 94549		
United States of America		
State (that is, country) of nationality:	State (that is, country) of residence:	
BE	US	
<input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet.		

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LAVAN, Brian
2020 Lawton Street
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State *(that is, country)* of residence:

US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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State *(that is, country)* of residence:

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US

State *(that is, country)* of residence:

US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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28073 Thorup Lane
Hayward, California 94542
United States of America

State *(that is, country)* of nationality:

CN

State *(that is, country)* of residence:

US



Further applicants are indicated on a continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative
 and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LOCKYER, Jean, M.
 TOWNSEND AND TOWNSEND AND CREW LLP
 Two Embarcadero Center, 8th Floor
 San Francisco, California 94111-3834
 United States of America

Telephone No.:

415.576.0200

Facsimile No.:

415.576.0300

Teleprinter No.:

Agent's registration No. with the Office

44,879

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION**Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description ☐ as originally filed
☒ as amended under Article 34

the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☒ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

- ☒ which is the language in which the international application was filed.
☐ which is the language of a translation furnished for the purposes of international search.
☐ which is the language of publication of the international application.
☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | | |
|--|---|-------|--------|
| 1. translation of international application | : | _____ | sheets |
| 2. amendments under Article 34 | : | 7 | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | _____ | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | _____ | sheets |
| 5. letter | : | 1 | sheet |
| 6. other (<i>specify</i>) | : | _____ | sheets |

For International Preliminary Examining Authority use only

received not received

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

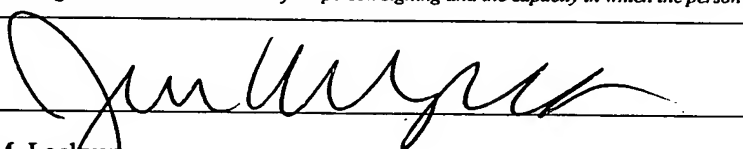
The demand is also accompanied by the item (s) marked below:

- | | |
|--|---|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 5. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> original separate signed power of attorney | 6. <input checked="" type="checkbox"/> sequence listing in computer readable form |
| 3. <input type="checkbox"/> original general power of attorney; | 7. <input type="checkbox"/> tables in computer readable form related to sequence listings |
| 4. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 8. <input checked="" type="checkbox"/> other (<i>specify</i>) Transmittal Letter; Article 34 Amendment with seven (7) substitute specification pages 6, 25, 45, 64, 65, 70 and 80; Sixty-two (62) pages of Sequence Listing, Statement and Diskette; Postcard |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

X


 Jean M. Lockyer
 TOWNSEND AND TOWNSEND AND CREW LLP
 USPTO Reg. No.: 44,879
 Applicants' Agent

For International Preliminary Examining Authority use only

- | | |
|--|---|
| 1. Date of actual receipt of DEMAND: | |
| 2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b): | |
| 3. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. | <input type="checkbox"/> The applicant has been informed accordingly. |
| 4. <input type="checkbox"/> The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5. | |
| 5. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82. | |

For International Bureau use only

Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand

For International Preliminary Examining Authority use only

International application No. PCT/US03/18046	Date stamp of the IPEA
Applicant's or agent's file reference 16325-136PC	
Applicant METABOLEX, INC. <i>et al.</i>	
CALCULATION OF PRESCRIBED FEES <div style="display: flex; justify-content: space-between; align-items: flex-end;"> <div style="width: 45%;"> 1. Preliminary examination fee </div> <div style="width: 45%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">490.00</div> <div style="border: 1px solid black; padding: 2px 5px; margin-left: 5px;">P</div> </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-end;"> <div style="width: 45%;"> 2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>) </div> <div style="width: 45%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">172.00</div> <div style="border: 1px solid black; padding: 2px 5px; margin-left: 5px;">H</div> </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-end;"> <div style="width: 45%;"> 3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box </div> <div style="width: 45%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">662.00</div> <div style="border: 1px solid black; padding: 2px 10px; margin-top: 2px;">TOTAL</div> </div> </div>	
MODE OF PAYMENT <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below) </div> <div style="width: 45%;"> <input type="checkbox"/> cash </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> cheque </div> <div style="width: 45%;"> <input type="checkbox"/> revenue stamps </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> postal money order </div> <div style="width: 45%;"> <input type="checkbox"/> coupons </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> bank draft </div> <div style="width: 45%;"> <input type="checkbox"/> other (<i>specify</i>): </div> </div>	
AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT <i>(This mode of payment may not be available at all IPEAs)</i> The IPEA/ <u>US</u> <div style="display: inline-block; vertical-align: top; margin-left: 10px;"> <input checked="" type="checkbox"/> is hereby authorized to charge the total fees indicated above to my deposit account. <input checked="" type="checkbox"/> (<i>this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit</i>) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account. </div> <div style="display: flex; justify-content: space-between; align-items: flex-end;"> <div style="width: 30%;"> <u>20-1430</u> Deposit Account Number </div> <div style="width: 30%;"> <u>5 January 2004</u> Date (day/month/year) </div> <div style="width: 30%; text-align: center;"> Signature </div> <div style="width: 10%; text-align: center;"> Jean M. Lockyer </div> </div>	

ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35 or SEQ ID NO:37. In some embodiments, the host cell is a human cell. In other embodiments, the host cell is a bacterium.

5 [0024] The present invention also provides isolated polypeptides comprising an amino acid sequence at least 70% identical to SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:34. In some embodiments, the polypeptide is SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID
10 NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36 or SEQ ID NO:38.

DEFINITIONS

[0025] "Insulin sensitivity" refers to the ability of a cell or tissue to respond to insulin.
15 Responses include, e.g., glucose uptake of a cell or tissue in response to insulin stimulation. Sensitivity can be determined at an organismal, tissue or cellular level. For example, blood or urine glucose levels following a glucose tolerance test are indicative of insulin sensitivity. Other methods of measuring insulin sensitivity include, e.g., measuring glucose uptake (*see, e.g., Garcia de Herreros, A., and Birnbaum, M. J. J. Biol. Chem.* 264, 19994-19999 (1989);
20 Klip, A., Li, G., and Logan, W.J. *Am. J. Physiol.* 247, E291-296 (1984)), measuring the glucose infusion rate (GINF) into tissue such as the skeletal muscle (*see, e.g., Ludvik et al., J. Clin. Invest.* 100:2354 (1997); Frias *et al., Diabetes Care* 23:64, (2000)) and measuring sensitivity of GLUT4 translocation (e.g., as described herein) in response to insulin.

[0026] "Activity" of a polypeptide of the invention refers to structural, regulatory, or
25 biochemical functions of a polypeptide in its native cell or tissue. Examples of activity of a polypeptide include both direct activities and indirect activities. Exemplary direct activities are the result of direct interaction with the polypeptide, e.g., enzymatic activity, ligand binding, production or depletion of second messengers (*e.g., cAMP, cGMP, IP₃, DAG, or Ca²⁺*), ion flux, phosphorylation levels, transcription levels, and the like. Exemplary indirect
30 activities are observed as a change in phenotype or response in a cell or tissue to a polypeptide's directed activity, e.g., modulating insulin sensitivity of a cell as a result of the interaction of the polypeptide with other cellular or tissue components.

against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

3. Column Chromatography

- 5 [0087] The proteins of interest can also be separated from other proteins on the basis of their size, net surface charge, hydrophobicity and affinity for ligands. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art.
- 10 [0088] Immunoaffinity chromatography using antibodies raised to a variety of affinity tags such as hemagglutinin (HA), FLAG, Xpress, Myc, hexahistidine (SEQ ID NO:45) (His), glutathione S transferase (GST) and the like can be used to purify polypeptides. The His tag will also act as a chelating agent for certain metals (e.g., Ni) and thus the metals can also be used to purify His-containing polypeptides. After purification, the tag is optionally removed by specific proteolytic cleavage.
- 15 [0089] It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

IV. DETECTION OF POLYNUCLEOTIDES OF THE INVENTION

- 20 [0090] Those of skill in the art will recognize that detection of expression of polynucleotides and polypeptides of the invention has many uses. For example, as discussed herein, detection of levels of polynucleotides and polypeptides of the invention in a patient is useful for diagnosing diabetes or a predisposition for at least some of the pathological effects of diabetes. Moreover, detection of gene expression is useful to identify modulators of expression of polynucleotides and polypeptides of the invention.
- 25 [0091] A variety of methods of specific DNA and RNA measurement that use nucleic acid hybridization techniques are known to those of skill in the art (*see*, Sambrook, *supra*). Some methods involve an electrophoretic separation (e.g., Southern blot for detecting DNA, and Northern blot for detecting RNA), but measurement of DNA and RNA can also be carried out in the absence of electrophoretic separation (e.g., by dot blot). Southern blot of genomic
- 30 DNA (e.g., from a human) can be used for screening for restriction fragment length

interleukin receptors, immunoglobulin receptors and antibodies, the cadherin family, the integrin family, the selectin family, and the like; *see, e.g.*, Pigott & Power, *The Adhesion Molecule Facts Book I* (1993)). Similarly, toxins and venoms, viral epitopes, hormones (*e.g.*, opiates, steroids, *etc.*), intracellular receptors (*e.g.*, which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

[0166] Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

[0167] Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly-Gly sequences of between about 5 and 200 amino acids (SEQ ID NO: 46). Such flexible linkers are known to those of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc., Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

[0168] Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent that fixes a chemical group to the surface that is reactive with a portion of the tag binder. For example, groups that are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups.

Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature (*see, e.g.*, Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, *e.g.*, peptides); Geysen *et al.*, *J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank and Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al.*, *Science*, 251:767-777 (1991); Sheldon *et al.*, *Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al.*, *Nature Medicine* 2(7):753759 (1996) (all describing

B/C	Diabetic Pre-Trog			Diabetic Post-Trog			Fold Change	Students t test	Gene name
	Mean Expr	SEM	N	Mean Expr	SEM	N			
B	1234	411	9	919	325	8	0.74	0.01	MAST205

Example 3

[0240] Real-time PCR analysis further shows that MAST205 is significantly over-expressed in muscle from diabetic individuals when compared to muscle from lean individuals.

Comparison	Expression Fold change	t test
Diabetes (19) / Lean (17)	1.45	0.001

Legend "Fold change" indicates fold change in MAT205 expression calculated as the ratio of mean obese expression/mean lean expression. Numbers in parentheses indicate the number of patient samples analyzed by real time PCR

Example 4

[0241] This example shows that MAST205b is up regulated in muscle of diabetics when compared to muscle of lean non-diabetic individuals. It also demonstrates that MAST205b is down-regulated in muscle of diabetics after 3 months of troglitazone treatment compared to before treatment.

[0242] PCR primers and Taqman Probe were designed to detect specifically the expression of MAST205b. The sequences of the primers was as follows:

Forward primer: 110F – ACAGCAGTCCTGGCACTCCTT (SEQ ID NO:39)

Reverse primer: 174R – GCGGTTACTTGTCCGACAACCTC (SEQ ID NO:40)

Probe133: TCCAGCCGCCCACTGCCG (SEQ ID NO:41)

Lean Pre-Trog	Lean Post-Trog	Diabetic Pre-Trog	Diabetic Post-Tro			
Relative Exp (%)	Relative Exp (%)	Relative Exp (%)	Relative Exp (%)	Fold Change (D-/L-)	Fold Change (D+/D-)	Gene name
100	88	197	111	1.97	0.56	MAST205b

Legend: "Pre-Trog" and "Post-Trog" refer to samples taken before and after 3 months of troglitazone treatment respectively. "Relative Exp" refers to the expression of the gene relative to the Lean Pre-Trog sample, which is set to 100%. D-/L- refers to the ratio of relative expression in Diabetic Pre-Trog to relative expression in Lean Pre-Trog. D+/L+ refers to the ratio of relative expression in Diabetic Post-Trog compared to relative expression in Diabetic Pre-Trog.

Example 5

[0243] This example shows that MAST205 is up regulated in muscle of diabetics when compared to muscle of lean non-diabetic individuals. It also demonstrates that MAST205 is

down-regulated in muscle of diabetics after 3 months of troglitazone treatment compared to before treatment.

[0244] PCR primers and Taqman Probe were designed to detect specifically the expression of MAST205. The sequences of the primers was as follows:

- 5 Forward primer: 717F – TTGGACAGTCTGCACCTTCTCTTA (SEQ ID NO:42)
Reverse primer: 801R – CGGTTACTTGTCCGACAAAAGC (SEQ ID NO:43)
Probe745: TGGCCTGAAGGACTTGAGCCTTCCAGCCCACTGCCG (SEQ ID NO:44)

Lean Pre-Trog	Lean Post-Trog	Diabetic Pre-Trog	Diabetic Post-Tro			
Relative Exp (%)	Relative Exp (%)	Relative Exp (%)	Relative Exp (%)	Fold Change (D-/L-)	Fold Change (D+/D-)	Gene name
100	98	242	66	2.42	0.27	MAST205

- 10 **Legend:** “Pre-Trog” and “Post-Trog” refer to samples taken before and after 3 months of troglitazone treatment respectively. “Relative Exp” refers to the expression of the gene relative to the Lean Pre-Trog sample, which is set to 100%. D-/L- refers to the ratio of relative expression in Diabetic Pre-Trog to relative expression in Lean Pre-Trog. D+/L+ refers to the ratio of relative expression in Diabetic Post-Trog compared to relative expression in Diabetic Pre-Trog.

15 *Example 6*

[0245] This example shows that MAST205 is up-regulated in skeletal muscle of DBA/2J mice fed a high fat diet. These mice became insulin resistant after 28 weeks on a 32% or 42% fat diet, compared to littermates fed a chow diet, as measured by IPIST.

	Chow Diet	32% Fat Diet	42% Fat Diet	Gene name
Mean Rel Exp (%)	118	153	170	Mouse MAST205
SEM	13	13	9	
N	5	5	5	
Fold Change	-	1.3	1.4	
Students T-test	-	0.09	0.008	

- 20 **Legend:** “Chow Diet” refers to standard mouse feed. “32% Fat Diet” and “42% Fat Diet” refer to mouse feed from in 32% or 42% of the calories in the diet are obtained from fat, respectively. “Mean Rel Exp (%)” refers to the average expression of the gene in muscles from 5 individual mice, relative to the expression in the muscle of a single mouse in the chow diet group.

25 colon Kruppel-like factor (CKLF)

[0246] Probe set MBXHUMMUS28900 detects CKLF nucleic acid sequences. Expression of transcripts encoding CKLF was higher in diabetic patients as compared to lean, non-diabetic patients in the gene profiling analysis described above.

SEQ ID NO:6 Human PAK1B polypeptide sequence

protein_id:gi1256422

MSNNGLDIQDKPPAPPMRNTSTMIGAGSKDAGTLNHGSKPLPPNPBEEKKKDRFYRSILPGDKTNKKKEKERPEI
SLPSDFEHTIHVGFDVAVTGEFTGMPEQWARLLQTSNITKSEQKKNPQAVLDVLEFYNSKKTSSNSQKYSFTDKSA
5 EDYNSSNALNVKAVSETPAVPPVSEDEDDDDDDATPPPVIAPRPEHTKSVYTRSVIEPLPVTPTRDVATSPISPT
ENNTTPPDALTLNTEKQKKKPKMSDEEILEKLRSIVSVGDPKKKYTRFEKIGQGASGTVYTAMDVATGQEVAIKQ
MNLQQQPKKELIINEILVMRENKNPNIVNYLDSYLVGDELWVMEYLAGGSLTDVVTETCMDEGQIAAVCRECLQ
ALESLSHNQVIHRDIKSDNILLGMDGSVKLTDFGFCAQITPEQSKRSTMVGTPTYWMAPEVVTRKAYGPKVDIWSL
GIMAIEMIEGEPYPYLNENPLRALYLIATNGTPELQNPEKLSAIFRDFLNRCLMDVEKRGSAKELLQHQLKIAK
10 PLSSLTPLIAAAKEATKNNH

SEQ ID NO:7 Human PAK1B splice variant nucleic acid sequence

accession:AF071884

coding sequence:12..1673

TGGTGGTGACAATGTCAAATAACGGCCTAGACATTCAAGACAAACCCCCAGCCCCTCCGATGAGAAATACCAGCA
15 CTATGATTGGAGTCGGCAGCAAAGATGCTGGAACCCCTAAACCATGGTTCTAAACCTCTGCCTCCAAACCCAGAGG
AGAAGAAAAAGAAGGACCGATTTTACCGATCCATTTTACCTGGAGATAAAACAAATAAAAAGAAAGAGAAAGAGC
GGCCAGAGATTTCTCTCCCTTCAGATTTTGAACACACAATTCATGTCGGTTTTGATGCTGTACAGGGGAGTTTA
CGGGAATGCCAGAGCAGTGGGCCCCGCTTGCTTCAGACATCAAATATCACTAAGTCGGAGCAGAAGAAAAACCCGC
AGGCTGTTCTGGATGTGTTGGAGTTTTACAACCTCGAAGAAGACATCCAACAGCCAGAAATACATGAGCTTTACAG
20 ATAAGTCAGCTGAGGATTACAATTCTTCTAATGCCTTGAATGTGAAGGCTGTGTCTGAGACTCCTGCAGTGCCAC
CAGTTTCAGAAGATGAGGATGATGATGATGATGATGCTACCCACCACAGTGATTGCTCCACGCCAGAGCACA
CAAAATCTGTATACACACGGTCTGTGATTGAACCACTTCCTGTCACTCCAACCTCGGGACGTGGCTACATCTCCCA
TTTCACCTACTGAAAATAACACCACTCCACCAGATGCTTTGACCCGGAATACTGAGAAGCAGAAGAAGAAGCCTA
AAATGTCTGATGAGGAGATCTTGGAGAAATTACGAAGCATAGTGAGTGTGGGCGATCCTAAGAAGAAATATACAC
25 GGTTTGAGAAGATTGGACAAGGTGCTTCAGGCACCGTGTACACAGCAATGGATGTGGCCACAGGACAGGAGGTGG
CCATTAAGCAGATGAATCTTCAGCAGCAGCCCAAGAAAGAGCTGATTATTAATGAGATCCTGGTCATGAGGGAAA
ACAAGAACCCAAACATTGTGAATTACTTGGACAGTTACCTCGTGGGAGATGAGCTGTGGGTGTTTATGGAATACT
TGGCTGGAGGCTCCTTGACAGATGTGGTGACAGAACTTGCATGGATGAAGGCCAAATTGCAGCTGTGTGCCGTG
AGTGTCTGCAGGCTCTGGAGTTCTTGCAATTCGAACCAGGTCATTACAGAGACATCAAGAGTGACAATATTCTGT
30 TGGGAATGGATGGCTCTGTCAAGCTAACTGACTTTGGATTCTGTGCACAGATAACCCAGAGCAGAGCAAACGGA
GCACCATGGTAGGAACCCATACTGGATGGCACCAGAGGTTGTGACACGAAAGGCCTATGGGCCCAAGTTGACA
TCTGGTCCCTGGGCATCATGGCCATCGAAATGATTGAAGGGGAGCCTCCATACCTCAATGAAAACCTCTGAGAG
CCTTGACCTCATTGCCACCAATGGGACCCAGAACTTCAGAACCCAGAGAAGCTGTGAGCTATCTTCCGGGACT
TTCTGAACCGCTGTCTCGAGATGGATGTGGAGAAGAGAGGTTTCAGCTAAAGAGCTGCTACAGGTGAGAAAACCTGA
35 GGTTCAGAGTGTCTAGTAACTTTTCCATGATAGCTGCATCAATTCCTGAAGATTGCCAAGCCCCCTCTCCAGCCTC
ACTCCACTGATTGCTGCAGCTAAGGAGGCAACAAAGAACAATCACTAAAACCACACTCACCCAGCCTCATTGTG
CCAAGCTCTGTGAGATAAATGCACATTTTCAGAAATTCCTGATGCCCTCTTCTCCTTGCTTCTCTCC
CATTCCTGATCTAGCACTCCTCAAGACTTTGATCCTTGGAACCGTGTGTCCAGCATTGAAGAGAACTGCAACT
GAATG
40

ATAGCTATGTAACTCGAAAATGAAAATGTTCTCTCTGCCTGTGTTTCTTTGAGTATCCAGTCAATGA
TCAGAACAAAGTAGCCCATTCAGTGATCTGTGAAATTGTTACACAAGGGTTGGCTACTCTATGCTAGCC
ATGGTTGATGTAAGAACTCAGTTTAATAGCATACTTGAGCAATCAGGGTAAAAGTGTTCCACCATGAA
CAAAGGTATCAAATCACAAACCTGGCTGACTGCAGTTGGATGTTAGAGGTCTGTTGTAAATAGTAGAA
5 CATAGATTGCAGACTGACTTGTTGATTTCTACTTTGGCAATAGCAATAAAGCACTTTGCAAAG

SEQ ID NO:26 Rat Protein C inhibitor polypeptide sequence

protein_id: 12621138

MRFFPILCLVLFFSHGVASRQRSHSKEKKKSKESSVGAVGTSRSDFAFRLYRALASEAPGQNVFFSP
10 MSVSMSLGLSLGSLKTKAQILEGLGLSLQQQEDMLHKGFFQQLLQQFSQPSDGLQLSLGSALFTDP
AVHIRDHFLSAMKTLYMSDMFSTNFGNPESAKKQINDYVAKKTNGKIVDLIKDLDSTHVMVVVNYIFF
KAKWQTAFFSSTNTHKMDFHVTPPKTIQVPMNREDIYSYILDQNISCTVVGIPYQGNTFALFILPSEG
KMKRVEDGLDERTLRNWLKMFTKRQLDLYLPKFSIEGTYKLEKILPKLGIQDIFTTHADLSGLTDHTN
IKLSEMVHKSMVEVDESGTTAAASTGILFTLRSARPSSLKVEFTRPFLVVIMDGTNLYFIGKVIQP
15

SEQ ID NO:27 Human MAST205b nucleic acid sequence

Nucleotide sequence Novel Variant MAST205b CDS:1-5073

ATGTTTTACCCACATCTGCTCCAGCCCTCTTCCTCACTAAAGTCCCATTAGTGCTGATTGTGCTTT
GGCTACTTCTCCTCTTGCCATTTTCCTGAACCCACGAGCCCACAGCAGTCCTGGCACTCCTTGTTCCA
20 GCCGCCCCTGCCGTGGAGTTGTCGGACAAGTAACCGCAAGAGCTTGATTGTGACCTCTAGCACATCA
CCTACACTACCACGGCCACACTCACCCTCCATGGCCACACAGGTAACAGTCCTTTGGACAGCCCCCG
GAATTTCTCTCCAAATGCACCTGCTCACTTTTCTTTTGTTCCTGCCCGTAGCCATAGCCACAGAGCTG
ACAGGACTGATGGGCGGCGCTGGTCTTTGGCCTCTTTGCCCTCTTCAGGATATGGAACCTAACACTCCT
AGCTCCACTGTCTCATCATCATGCTCCTCACAGGAAAAGCTGCATCAGTTGCTTTTCCAGCCTACAGC
25 TGATGAGCTGCACTTTTTGACGAAGCATTTCAGCACAGAGAGCGTACCAGATGAGGAAGGACGGCAGT
CCCCAGCCATGCGGCCTCGCTCCCGGAGCCTCAGTCCCGGACGATCCCCAGTATCCTTTGACAGTGAA
ATAATAATGATGAATCATGTTTACAAAGAAAGATTCCCAAAGGCCACCGCACAAATGGAAGAGCGACT
AGCAGAGTTTATTTCTCCAACACTCCAGACAGCGTGCTGCCCTTGGCAGATGGAGCCCTGAGCTTTA
TTCATCATCAGGTGATTGAGATGGCCCGAGACTGCCTGGATAAATCTCGGAGTGGCCTCATTACATCA
30 CAATACTTCTACGAACTTCAAGAGAATTTGGAGAACTTTTACAAGATGCTCATGAGCGCTCAGAGAG
CTCAGAAGTGGCCTTTGTGATGCAGCTGGTGAAAAAGCTGATGATTATCATTGCCCCGCCAGCACGTC
TCCTGGAATGCCTGGAGTTTGACCCTGAAGAGTTCTACCACCTTTTAGAAGCAGCTGAGGGCCACGCC
AAAGAGGGACAAGGGATTAAATGTGACATTCCCCGCTACATCGTTAGCCAGCTGGGCCTCACC CGGA
TCCCCTAGAAGAAATGGCCAGTTGAGCAGCTGTGACAGTCCTGACACTCCAGAGACAGATGATTCTA
35 TTGAGGGCCATGGGGCATCTCTGCCATCTAAAAAGACACCCTCTGAAGAGGACTTCGAGACCATTAAAG
CTCATCAGCAATGGCGCCTATGGGGCTGTATTTCTGGTGCGGCACAAGTCCACCCGGCAGCGCTTTGC
CATGAAGAAGATCAACAAGCAGAACCTGATCCTACGGAACCAGATCCAGCAGGCCTTCGTGGAGCGTG